

Serial No.: 09/981,289

Filed: October 15, 2001

**CLAIMS LISTING:**

1. (Currently Amended) A non-naturally occurring variant TNF- $\alpha$  ~~protein trimer~~ comprising at least one non-naturally occurring variant TNF- $\alpha$  protein, wherein said TNF- $\alpha$  protein comprises an amino acid sequence that has at least one amino acid substitution as compared to the ~~wild-type a naturally occurring human TNF- $\alpha$  monomer~~ sequence, ~~wherein said variant TNF- $\alpha$  trimer will interact with the wild-type TNF- $\alpha$  monomer to form mixed trimers incapable of activating receptor signaling.~~
2. (Currently Amended) A non-naturally occurring variant TNF- $\alpha$  protein trimer according to claim 1, wherein said non-naturally occurring variant TNF- $\alpha$  protein has from 3 to 5 amino acid substitutions as compared to a naturally occurring human TNF- $\alpha$  monomer sequence.
3. (Currently Amended) ~~The~~ A non-naturally occurring variant TNF- $\alpha$  protein trimer according to claim 1, wherein said substitutions are selected from the ~~group of substitutions~~ consisting of K112D, Y115T, D143K, D143R, and Y115L.
4. (Canceled)
5. (Canceled)
6. (Canceled)
7. (Canceled)
8. (Canceled)
9. (Canceled)
10. (Canceled)
11. (Canceled)
12. (Canceled)
13. (Currently Amended) ~~The~~ A non-naturally occurring variant TNF- $\alpha$  protein trimer according to claim 3, wherein said substitution ~~consists of~~ Y115T (SEQ ID NO: 20).
14. (Currently Amended) ~~The~~ A non-naturally occurring variant TNF- $\alpha$  protein trimer according to claim 1, wherein said amino acid substitutions are selected from amino acid residues at positions 21, 30, 31, 32, 33, 35, 65, 66, 67, 111, 112, 115, 140, 143, 144, 145, 146 and 147.
15. (Currently Amended) The non-naturally occurring variant TNF- $\alpha$  protein trimer according to claim 14, wherein at least one of said amino acid substitutions are selected from the group of

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substitutions consisting of D143E, D143N, D143S, A145R, A145K, A145E, E146K, E146R and A84V.

16. (Currently Amended) A ~~method of recovering a~~ non-naturally occurring variant TNF- $\alpha$  ~~protein~~ trimer comprising an amino acid sequence that has at least one amino acid substitution as compared to ~~the wild-type~~ a naturally occurring human TNF- $\alpha$  sequence, wherein said variant TNF- $\alpha$  trimer will ~~preferentially interact with the wild-type~~ a naturally occurring TNF- $\alpha$  to form mixed trimers incapable of activating receptor signaling, ~~from a host cell.~~

17. (New) A non-naturally occurring variant TNF- $\alpha$  trimer according to claim 16, wherein said mixed trimers are formed *in vivo*.

18. (New) A non-naturally occurring variant TNF- $\alpha$  trimer according to claim 1, wherein said variant TNF- $\alpha$  trimer will interact with a naturally occurring human TNF- $\alpha$  monomer to form mixed trimers incapable of activating receptor signaling.

19. (New) A non-naturally occurring variant TNF- $\alpha$  trimer.

20. (New) A non-naturally occurring variant TNF- $\alpha$  trimer according to claim 19, wherein said trimer comprises three non-naturally occurring TNF- $\alpha$  proteins.

21. (New) A non-naturally occurring variant TNF- $\alpha$  trimer according to claim 20, wherein said trimer comprises at least one different non-naturally occurring TNF- $\alpha$  proteins.

22. (New) A non-naturally occurring variant TNF- $\alpha$  trimer according to claim 20, wherein said trimer comprises at least two different non-naturally occurring TNF- $\alpha$  proteins.

23. (New) A non-naturally occurring variant TNF- $\alpha$  trimer according to claim 20, wherein said trimer comprises at least three different non-naturally occurring TNF- $\alpha$  proteins.

24. (New) A non-naturally occurring variant TNF- $\alpha$  trimer according to claim 21 or 22, wherein said trimer comprises at least one naturally occurring human TNF- $\alpha$  protein.

25. (New) A non-naturally occurring variant TNF- $\alpha$  trimer according to claim 1-23, wherein said variant TNF- $\alpha$  trimer further comprises at least one covalent modification.

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26. (New) A non-naturally occurring variant TNF- $\alpha$  trimer according to claim 25, wherein said covalent modification comprises a polyethylene glycol molecule.

27. (New) A method of antagonizing naturally occurring human TNF- $\alpha$  comprising administering a non-naturally occurring variant TNF- $\alpha$  trimer, wherein said trimer further comprises at least one amino acid sequence having at least one amino acid substitution as compared to the wild type TNF- $\alpha$  sequence.

D/ 28. (New) A non-naturally occurring variant TNF- $\alpha$  protein comprising an amino acid sequence that has at least one amino acid substitution as compared to the wild-type human TNF- $\alpha$  sequence, wherein said variant TNF- $\alpha$  protein will interact with a naturally-occurring human TNF- $\alpha$  to form mixed trimers incapable of activating receptor signaling, wherein said substitutions are selected from the group consisting of 21, 30, 31, 32, 35, 66, 111, 112, 140, K112D, Y115T, D143K, D143R, and Y115I.

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